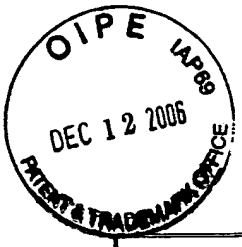
Receipt



PATENT
ATTORNEY DOCKET NO. 50026/054001

Certificate of Mailing: Date of Deposit: December 8, 2006

I hereby certify under 37 C.F.R. § 1.8(a) that this correspondence is being deposited with the United States Postal Service as **first class mail** with sufficient postage on the date indicated above and is addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Collette D'Amico
Printed name of person mailing correspondence

Collette R. D'Amico
Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Hamada et al.	Art Unit:	1653
Serial No.:	10/546,000	Examiner:	Not Yet Assigned
371(c) Date:	June 14, 2006	Customer No.:	21559
Title:	METHODS FOR TREATING ISCHEMIC DISEASES		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST TO CORRECT FILING RECEIPT

Applicants request that the enclosed filing receipt be corrected as follows.

Please change the title of the application listed on the Filing Receipt as follows:

"Method for treating ischemic diseases"

to read:

"Methods for treating ischemic diseases"

Enclosed is a copy of the incorrect filing receipt indicating the change to be made. In support of this change, enclosed is a copy of the Preliminary Amendment filed with the application on August 18, 2005 in which the title was amended.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 8 December 2006

Jan N. Tittel, Ph.D.
Reg. No. 52,290

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPL NO.	FILING OR 371 (c) DATE	ART UNIT	FIL FEE REC'D	ATTY.DOCKET NO	DRAWINGS	TOT CLMS	IND CLMS
10/546,000	06/14/2006	1653	2190	50026/054001	21	19	4

CONFIRMATION NO. 8449

21559
CLARK & ELBING LLP
101 FEDERAL STREET
BOSTON, MA 02110

DE-DOCKETED

FILING RECEIPT

OC000000021132206

Date Mailed: 11/14/2006

Receipt is acknowledged of this regular Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please mail to the Commissioner for Patents P.O. Box 1450 Alexandria Va 22313-1450. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Hirofumi Hamada, Sapporo-shi, JAPAN;
Yoshinori Ito, Sapporo-shi, JAPAN;
Kazuhiro Takahashi, Kushire-shi, JAPAN;
Masayuki Morikawa, Sapporo-shi, JAPAN;

Assignment For Published Patent Application

DNAVEC Research, Inc., Tuskuba-shi, JAPAN

Power of Attorney: The patent practitioners associated with Customer Number **21559**.

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/JP04/00957 01/30/2004

Foreign Applications

JAPAN 2003-040806 02/19/2003

If Required, Foreign Filing License Granted: 11/06/2006

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US10/546,000

Projected Publication Date: 02/15/2007

Non-Publication Request: No

Early Publication Request: No

Title Methods

~~Method~~ for treating ischemic diseases

Preliminary Class

435

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

COPY

PATENT
ATTORNEY DOCKET NO. 50026/054001

Certificate of Mailing: Date of Deposit: August 18, 2005

I hereby certify under 37 C.F.R. § 1.10 that this correspondence is being deposited with the United States Postal Service as **Express Mail Post Office to Addressee (Express Mail No. EV 450824522 US)** with sufficient postage on the date indicated above and is addressed to: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Guy Beardsley
Printed name of person mailing correspondence

Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Hamada et al.	Art Unit:	Not Yet Assigned
Serial No.:	Not Yet Assigned	Examiner:	Not Yet Assigned
Filed:	August 18, 2005	Customer No.:	21559
Title:	METHODS FOR TREATING ISCHEMIC DISEASES		

Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Prior to examination of the above-captioned application, kindly amend the application as follows.

COPY

Amendments to the specification

Please note that all page numbers and line numbers herein refer to the concurrently filed English language translation of the Japanese language specification.

Please amend the title as follows.

~~METHOD~~ METHODS ~~OF~~ FOR TREATING ISCHEMIC ~~DISEASE~~ DISEASES

Please insert the following heading and paragraph at page 1, line 5.

Cross-Reference to Related Applications

This application is the U.S. national stage application of International Application Number PCT/JP2004/000957, filed January 30, 2004, which, in turn, claims the benefit of Japanese Patent Application Serial Number 2003-040806, filed February 19, 2003.

Please amend the paragraph starting at page 6, line 17 as follows.

[3] the method according to [1] or [2], wherein ~~angiopoietin-1 or~~ the vector encoding angiopoietin-1 is a viral vector ~~encoding angiopoietin-1~~;

Please amend the paragraph starting at page 6, line 22 as follows.

[6] the method according to [1] or [2], wherein ~~angiopoietin-1 or~~ the vector encoding angiopoietin-1 is a naked DNA;

COPY

Please amend the paragraph starting at page 6, line 24 as follows.

[7] the method according to any one of [1] to [6], wherein ~~angiopoietin-1 or~~ the vector encoding angiopoietin-1 is a vector that drives angiopoietin-1 expression using CA promoter or a promoter having a transcriptional activity equivalent to or higher than that of said CA promoter;

Please amend the paragraph starting at page 17, line 36 as follows.

In the case of a virus, ~~the dosage can be administered~~ administration may take place, for example, at one or more sites (for example, two to ten sites) in the surviving muscle (skeletal muscle, cardiac muscle, or such) surrounding the ischemic site. In the case of an adenovirus, the dosage preferably ranges from, for example, 10^{10} to 10^{13} pfu/body, and more preferably 10^{11} to 10^{13} pfu/body. The dosage of a minus-strand RNA virus preferably ranges from, for example, $2x\ 10^5$ to $5x\ 10^{11}$ CIU. A naked DNA can be administered at one or more sites (for example, two to ten sites) in the surviving muscle surrounding the ischemic site. The injection dosage per site preferably ranges from, for example, 10 μg to 10 mg, and more preferably 100 μg to 1 mg. When performing an *ex vivo* administration of cells into which a vector has been introduced, the vector is introduced into the target cells (for example, in a test tube or dish) *ex vivo*, for example, at a multiplicity of infection (MOI) of 1 to 500. In the present invention, minus-strand RNA viral vectors have been found to introduce foreign genes into mesenchymal cells with exceedingly high efficiency. Accordingly, when mesenchymal cells are used in an *ex vivo*

administration, it is preferable to use a minus-strand RNA viral vector to introduce genes into the mesenchymal cells. When Ang-1 gene-introduced cells are used, for example, 10^5 to 10^9 cells, and preferably 10^6 to 10^8 cells can be transplanted to ischemic tissues. When a protein preparation is used, it may be administered at one or more sites (for example, two to ten sites) in the surviving muscle surrounding the ischemic site. The dosage preferably ranges from, for example, 1 μg/kg to 10 mg/kg, and more preferably 10 μg/kg to 1 mg/kg. Alternatively, the vector or the protein preparation may be administered, for example, several times (one to ten times) to the artery that leads to the ischemic tissue (for example, the coronary artery of an ischemic heart). In such cases, when a protein preparation is used, the dosage per site preferably ranges from, for example, 1 μg/kg to 10 mg/kg, and more preferably 10 μg/kg to 1 mg/kg. A vector or protein preparation may be administered intravenously several times (one to ten times) or it may be administered continuously. In such cases, when a protein preparation is used, the total dosage preferably ranges from, for example, 1 μg/kg to 10 mg/kg, and more preferably 10 μg/kg to 1 mg/kg. When a vector is used, it may be administered at the same dosage as described above for the intramuscular injection. See, Freedman SB *et al.* Ann Intern Med 136:54-71 (2002), for dosage.

Please amend the paragraph starting at page 24, line 3 as follows.

In another embodiment, the cell fraction contains mesodermal ~~liver~~ stem cells with SH2(+), SH3(+), SH4(+), CD29(+), CD44(+), CD14(-), CD34(-), and CD45(-).

Please amend the paragraph starting at page 25, line 32 as follows.

A specific example involves first mixing a solution (2 ml L-15 + 3 ml Ficoll) with a bone marrow fluid (5 μl to 10 μl) collected from vertebrate, and centrifuging the resulting mixture at 2,000 rpm for 15 minutes to separate a mononuclear cell fraction (about 1 ml). The cells were then washed by mixing the mononuclear cell fraction with a culture solution (2 ml of DMEM), and centrifuging the mixture at 2,000 rpm for 15 minutes for the second time. The supernatant is then discarded, and the precipitated cells are collected. In addition to the thighbone, the cell fractions of the present invention have sources including the sternum, and the iliac bone which constitutes the pelvis. The cell fractions can be obtained not only from these bones but also from other large bones. The cell fractions can be also collected from bone marrow fluids or cord blood ~~stored in~~ provided by bone-marrow banks. When cord blood cells are used, the cell fraction can be collected from cord blood ~~stored in~~ provided by bone-marrow banks.

Please amend the paragraph starting at page 28, line 22 as follows.

The present invention also relates to methods for producing genetically modified oral squamous cells, comprising the step of contacting oral squamous cells with a minus-strand RNA viral vector. Furthermore, the present invention also relates to methods for producing genetically modified macrophages, comprising the step of contacting macrophages with a minus-strand RNA viral vector that carryies a gene. The present

invention also relates to methods for producing genetically modified dendritic cells, comprising the step of contacting dendritic cells with a minus-strand RNA viral vector that carryies a gene. It was found that a minus-strand RNA viral vector can introduce genes into oral squamous cells, macrophages, and dendritic cells with an exceedingly higher efficiency, compared with an adenoviral vector which is generally expected to express high levels of an introduced gene. Thus, a minus-strand RNA viral vector is highly useful to introduce genes into oral squamous cells (including oral squamous carcinoma cells), macrophages, and dendritic cells. Specifically, the present invention relates to (i) methods for producing genetically modified oral squamous cells, comprising the step of contacting oral squamous cells with a minus-strand RNA viral vector carrying a gene; (ii) methods for producing genetically modified macrophages, comprising the step of contacting ~~dendritic cells~~ macrophages with a minus-strand RNA viral vector carrying a gene, and (iii) methods for producing genetically modified ~~macrophages~~ dendritic cells, comprising the step of contacting dendritic cells with a minus-strand RNA viral vector carrying a gene. Furthermore, the present invention also relates to genetically modified cells produced by the methods described above. Such a genetic modification of cells is useful for regulating the immune system in gene therapy for oral squamous cell carcinoma and gene therapy for cancers and immune diseases.

Please amend the paragraph starting at page 30, line 24 as follows.

Fig. 8 presents graphs showing the expression of LacZ in rat skeletal muscles (A)

and hearts (B) as a result of the injection of naked DNA. ~~An indicated amount of plasmid (20 μg) was~~ 20 μg of plasmid were injected into the femoral muscle of the lower limb or cardiac apex (n=4). β-gal activity was assayed using Galacto-light plus kit four days after the plasmid injection and is shown as ng activity of LacZ in muscle or heart. Bar represents the standard error.

Please amend the paragraph starting at page 32, line 12 as follows.

Human VEGF gene was obtained by PCR cloning of cDNA derived from a human glioma cell line U251. The nucleotide sequence of the obtained VEGF gene was confirmed by BigDye Terminator method (Perkin-Elmer). Human Ang1 gene was PCR cloned from cDNA derived from human bone marrow cells, and the nucleotide sequence was confirmed by the same procedure described above. Comparison of the determined nucleotide sequence of the Ang1 gene with that registered under the accession number U83508 in GenBank suggested that they are identical, except that the nucleotide A at position 933 had been replaced with G. Despite of the nucleotide substitution, the amino acid sequence of Ang1 protein is identical to that of U83508 in GenBank. The cloned VEGF and Ang1 cDNAs were individually inserted between the restriction sites EcoRI and BglII of a pCAcc vector (WO 02/100441; Ito., Y., *et al.* (2002) Mol Ther. 5: S162) derived from pCAGGS (Niwa, H. *et al.* (1991) Gene. 108: 193-199). Thus, the respective VEGF and Ang1 expression vectors, pCAhVEGF and pCAhAng1, were prepared. Adenoviruses expressing either VEGF or Ang1 were prepared by the COS-TPC method

developed by Saito *et al.* (Miyake, S., Proc. Natl. Acad. Sci. USA 93: 1320-1324 (1996)). The plasmids of pCAhVEGF and pCAhAng1 were digested with a restriction enzyme ClaI. The resulting gene expression units, each comprising a VEGF or Ang1 cDNA and a CA promoter, were inserted into the ClaI restriction site of the cosmid pAxcw (Nakamura, T. *et al.* (2002) Hum Gene Ther. 13: 613-626) comprising a portion of the adenovirus type 5 gene, to produce ~~pAxCAhVEGF/Ang1~~ pAxCAhVEGF and pAxCAhAng1. A DNA-terminal protein complex (TPC) comprising ~~pAxCAhVEGF/Ang1~~ pAxCAhVEGF or pAxCAhAng1 and full-length adenovirus type 5 was digested with a restriction enzyme EcoT22I, and then the product was introduced into 293 cells by a calcium phosphate coprecipitation method. Plaques which contain the modified adenovirus were then harvested (Graham, F. L. and A. J. van der Eb. (1973) Virology. 52: 456-467). The adenovirus from each plaque was confirmed based on its restriction enzyme digestion pattern. Furthermore, it was confirmed by PCR that the viruses were not contaminated with the wild-type virus. Thus, the respective adenoviral vectors AxCAhVEGF and AxCAhAng1 for expressing VEGF and Ang1 were prepared. The adenoviruses to be used for generating a rat model of myocardial infarction were purified by ultracentrifugation in a CsCl discontinuous density gradient and dialyzed against 10% glycerol/PBS (Kanegae, Y., *et al.* (1995) Nucleic Acids Res. 23: 3816-3821). The concentrations (optical density units/ml, opu/ml) of the purified adenoviral vectors were measured by the A_{260} in the presence of 0.1% SDS and determined by using the following formula (Nyberg-Hoffman, C. *et al.* (1997) Nat Med. 3: 808-811):

opu = A_{260} x (1.1×10^{12})

Please amend the paragraph starting at page 35, line 20 as follows.

Ang1 expression in the hearts, into which the vector had been introduced, was also examined by RT-PCR (Fig. 3). The hearts were excised five days after the adenoviral vector-mediated gene introduction ($1x\ 10^{10}$ opu/heart). Total RNAs were extracted from the left ventricular cardiac muscle using RNeasy Kit (Qiagen KK, Tokyo, Japan). To avoid DNA contamination in the total RNAs of cardiac muscle, the samples were treated with DNaseI using RNase-free DNase Set (Qiagen) according to the attached instruction. The first cDNA strands were synthesized from the total RNAs by a random priming method using a random primer mixture (Invitrogen, Carlsbad, CA) and Superscript™ II (Invitrogen). The human Ang1-specific mRNA transcribed from the adenoviral vector was detected using a forward primer that is human Ang1-specific and a reverse primer for the rabbit β-globulin located at the terminator site of the Ang1 expression unit. The internal control rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was also detected by RT-PCR. The human Ang1 forward primer, rabbit β-globulin reverse primer, and rat GAPDH primer are shown below.

Please amend the paragraph starting at page 35, line 33 as follows.

Human Ang1 primer:

Forward primer: 5'-CAGAGGCAGTACATGCTAAGAATTGAGTTA-3' (SEQ ID

NO: 6)

Rabbit β-globulin primer:

Reverse primer: 5'-AGATGCTCAAGGGGCTTCATGATG-3' (SEQ ID NO: 7)

~~Rabbit~~ Rat GAPDH primer:

Forward primer: 5'-TATTGGGCGCCTGGTCACCA-3' (SEQ ID NO: 8)

Reverse primer: 5'-CCACCTTCTTGATGTCATCA-3' (SEQ ID NO: 9)

Please amend the paragraph starting at page 36, line 4 as follows.

Thirty cycles of PCR were performed, and human Ang1 mRNA and rat GAPDH mRNA were detected. The resulting PCR products were separated on a 2% agarose gel. Total RNA was extracted from HeLa cells that had been infected with AxCAhAng1 at 100 opu/cell, and used as a positive control for the human Ang1 mRNA.

Please amend the paragraph starting at page 37, line 20 as follows.

The vascular densities in the infarcted site and the peri-infarct myocardium of the infarcted hearts were found to have decreased as compared with the normal hearts (Fig. 4). When VEGF or Ang1 was administered using an adenovirus, the vascular density was significantly increased in the infarcted site and the peri-infarct myocardium. In particular, the vascular density in the peri-infarct region, which is close to the site of gene administration, was increased to a level higher than in normal heart muscles (the vascular density in the peri-infarct myocardium: 644±96 /mm^2 in the Ang1-treated group, 350±79

/mm^2 in the physiological saline-treated group ($p<0.01$ vs the Ang1-treated group), 332±127 /mm^2 in the AxCAZ3-treated group ($p<0.01$ vs the Ang1-treated group), or 402±121 /mm^2 in the sham- operated group). Hemangioma was not found in the Ang1-treated group, either macroscopically or microscopically. Interestingly, the physiological saline-treated group and AxCAZ3-treated group showed a reduction in the number of blood vessels in the interventricular septum distant from the site of gene administration four weeks after myocardial infarction (341±60 /mm^2 and 367±113 /mm^2, respectively). The decrease in the number of septal blood vessels was suppressed by the administration of Ang1 gene (461±100 /mm^2) or VEGF gene (483±46 /mm^2 in the sham-operated group). Fig. 5 shows immunostaining of vascular endothelia with the anti-CD34 MoAb. Micro vessels with diameters of 10 μm or less, as well as blood vessels with diameters of 10 μm or more, were found in the Ang1 gene-administered group (for every sample, many α-SMA-positive blood vessels were found in the left ventricular region of the infarcted hearts treated with Ang1; 38.9±7.35 /mm^2 in the septal region, 38.9±4.81 /mm^2 in the boundary region, and 112±26.1 /mm^2 in the infarcted region). In every group except for the Ang1-treated group, there was no significant alteration in the density of α-SMA-positive blood vessels with diameters greater than 10 μm (19 to 22 /mm^2). In addition, it was found that administration of Ang1 alone increased the vascular density to the same extent as the administration of the VEGF gene (Fig. 4).

Please amend the paragraph starting at page 38, line 23 as follows.

Total left ventricle (LV) area (mm^2), ~~infarction~~ infarcted area (mm^2), septal wall thickness (mm), ~~infarction~~ infarct wall thickness (mm), epicardial and endocardial ~~circumference~~ circumferences of LV (mm), and epicardial and endocardial ~~infarction~~ infarct ~~length~~ lengths (mm).

Please amend the paragraph starting at page 38, line 26 as follows.

From these results, evaluation was performed using the following formulas:

% ~~infarction~~ infarct size = infarcted region / total LV area x 100

% Ant/septal wall thickness = anterior wall (infarct) thickness / septal wall thickness x 100

~~Viable~~ Surviving LV area = (total LV myocardial area) - (infarcted myocardial area);

%endocardial infarct length = endocardial infarct length / endocardial circumference of LV x 100;

%epicardial infarct length = epicardial infarct length / epicardial circumference of LV x 100;

Please amend the paragraph starting at page 38, line 33 as follows.

As shown in Fig. 5, clear signs of cardiac failure are observed in the infarcted cardiac muscles, including thinning of the myocardial walls in the infarct and throughout the surviving left ventricular myocardium, and tendency of left ventricular lumen

enlargement. As shown in Table 2, when compared with those of the control group, the infarct region was reduced significantly (% ~~infarction~~ infarct size) and the mass of surviving myocardium increased significantly (%~~viable~~ surviving LV area) in the Ang1 gene-administered group. Thus, it was clearly shown that Ang1 has an effect on surviving myocardium, as well as an effect of reducing the size of myocardial infarct. The % infarct thickness parameter, which reflects the thickness of an infarcted wall, was also found to have significantly increased in the Ang1- administered group.

Please amend the paragraph starting at page 43, line 10 as follows.

Twenty μg of naked plasmid in 0.1 ml of 0.9% physiological saline was injected into the skeletal muscles or hearts of Lewis rats (male, 8-week old, 250 to 300 g weight; Sankyo Labo Service (Tokyo, Japan)) using a 1 ml syringe with a 27G injection needle. AxCAZ3 adenoviral particles (1x 10^{10}, 5x 10^{9}, and 1x 10^{9} OPU) in 0.1 ml of 0.9% physiological saline were also injected into the hearts. For the injection into skeletal muscles, the hind leg skin was incised by 2 cm long to facilitate injection into the femoral muscles (Wolff, J.A. *et al.*, Science 1990; 247: 1465-1468). For the injection into the heart, the left chest was opened and the naked plasmids or adenovirus particles were injected into the cardiac apex (Lin, H. *et al.*, Circulation 1990; 82: 2217-2221). After the injection, the incision wounds were sutured with silk sutures.

Please amend the paragraph starting at page 44, line 21 as follows.

LacZ expression levels in the heart were compared between the CA promoter-based plasmid vector and the adenoviral vector. The adenoviral vector AxCAZ3 was injected into the cardiac apex at various doses (1x 10^{10}, 5x 10^9, and 1x 10^9 OPU) (n= 4). After five days, the LacZ expression level in the heart, into which AxCAZ3 had been injected, was compared with that in the cardiac tissues into which 20 μg of pCAZ2 had been injected. The result showed that the average expression level of the introduced gene in the heart, mediated by 20 μg of pCAZ2, was found to be comparable to that mediated by 6.0x 10^9 OPU of AxCAZ3 (Fig. 9).

Please amend the paragraph starting at page 44, line 28 as follows.

pcDNA3LacZ (20μg), pCAZ2 (20μg), or 5x 10^9 OPU of AxCAZ3 was injected into the cardiac muscle, followed by X-gal staining. LacZ-positive muscle cells were found in all the samples from the tested groups. There were almost no LacZ-positive cells in areas surrounding the injection site of the heart samples into which pcDNA3LacZ had been injected. In contrast, when pCAZ2 was used, LacZ-positive myocardial cells which have high expression levels of the gene were found sporadically in the areas surrounding the injection site. The expression level and pattern of the introduced gene in cardiac tissues, into which 5x 10^9 OPU of AxCAZ3 had been injected, were similar to those in the tissues where pCAZ2 had been injected. As demonstrated above, the direct administration of the plasmids results in exceedingly efficient expression of the

introduced genes in the cardiac muscle, and achieves a high-level expression almost equivalent to that with the adenoviral vector, especially when CA promoter is used.

Please amend the paragraph starting at page 47, line 33 as follows.

The results are shown in Fig. 12. As commonly known, the Ad vector-introduced foreign gene was mainly expressed in the liver after intravenous administration. The SeV vector, on the other hand, was different from the AdV; when the SeV vector was used, the reporter gene was expressed in the lung, heart, and spleen, but hardly expressed in the liver. The results shown in the Figure were obtained by using extracts from the whole organs ("lung" indicates the right lung and "kidney" indicates the right kidney). The expression level increases in the order of heart < spleen < lung with considerations given to the organ weight. The organ distribution pattern of gene expression after the intramyocardial administration was found to be nearly the same as that after the intravenous administration. Therefore, organs targeted for gene expression in other organs as a result of the SeV vector overflow were revealed to be the lung and spleen.

Please amend the paragraph starting at page 49, line 11 as follows.

Total left ventricle (LV) area (mm^2), ~~infarction~~ infarcted area (mm^2), septal wall thickness (mm), ~~infarction~~ infarct wall thickness (mm), epicardial and endocardial ~~circumference~~ circumferences of LV (mm), and epicardial and endocardial ~~infarction~~ infarct ~~length~~ lengths (mm).

Please amend the paragraph starting at page 49, line 14 as follows.

The size was then evaluated based on the results using the following formula.

%~~infarction~~ infarct size = infarcted region / total LV area x 100

%~~infarction~~ infarct thickness = anterior wall (~~infarction~~ infarct) thickness / septal wall thickness x 100

~~Viable~~ Surviving LV area = (total LV myocardial area) - (infarction myocardial area);

Please amend the paragraph starting at page 50, line 3 as follows.

Lewis rats (eight-week old, male, and about 300 g weight) were anesthetized by inhalation of diethyl ether and intramuscular injection of 40 mg/kg ketamine and 4 mg/kg xylazine via an upper limb. After shaving both lower limbs, the abdominal region and the left inguinal region were incised, and the ~~right~~ left iliac artery, ~~right~~ left femoral artery, and their branches were all exposed. After the ~~right~~ left iliac artery and its branches were ligated, the left femoral artery was also ligated at its origin, immediately before the bifurcation into the popliteal and saphenous arteries. Furthermore, all other branches of the left femoral artery were identified and ligated, and then the left femoral artery was removed surgically. In the operation, 5x 10^7 CIU of the SeV vector was administered at two sites on the rectus femoris muscle using a 30G needle. After confirming that there were no hemorrhages, the surgical wound was sutured to complete the operation. In the null group, 5x 10^7 CIU of SeVNull was injected instead of SeVAng1, and in the negative control group, 0.9% physiological saline was injected.

Please amend the paragraph starting at page 50, line 15 as follows.

The blood flow analysis was carried out using Laser-Doppler imaging as described below. The blood flow in the lower limb was measured continuously over two weeks after ischemia (on day 1, day 3, day 7, and day ~~148~~ 14 after ischemia) using a Laser Doppler system (Moor LDI, Moor Instruments, Devon, United Kingdom). The rats were anesthetized by inhalation of ether, and then further anesthetized and sedated with ketamine (25 mg/kg) and xylazine (2 mg/kg). The rats were kept at 37°C for 10 minutes and then analyzed for blood flow. The continuous blood flow measurements were carried out at the identical spots in the same rats. The resulting blood flow images were analyzed to estimate the mean blood flow in the feet and gastrocnemius regions of both lower limbs. To reduce the influence of measurement conditions, the blood flow ratio of ischemic side (left lower limb) to normal side (right lower limb) (tissue blood flow ratio: blood flow on the ischemic side/blood flow on the normal side) was then calculated.

Please amend the paragraph starting at page 51, line 8 as follows.

Rat ~~cardiac~~ mesenchymal stem cells (MSC) were separated from Lewis rat thighbones according to the previous report (Tsuda, H., T. Wada, *et al.* (2003) Mol Ther 7(3): 354-65). Both ends of the thighbones were cut off, and bone marrows were collected by flushing the bones with 10% FBS-containing Dulbecco's modified Eagle's medium (DMEM) with an injector. The resulting bone marrow suspension was passed through 18, 20, and 22G needles successively to prepare a bone marrow cell suspension.

The obtained bone marrow cells were plated at a cell density of 5x 10^7 nucleated cells/10 cm culture dish and cultured for 4 days in culture medium (DMEM containing 10%FBS, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin, and 2 mM L-glutamine). The culture medium was changed every 3 to 4 days to remove floating cells. The adherent cells were passaged and used as rat MSCs.

Please amend the paragraph starting at page 53, line 1 as follows.

(1) ~~Cultured cell line~~ Human cervical cancer cell

Please replace the Sequence Listing found in the English language translation of PCT/JP2004/000957 with the enclosed paper copy of the Sequence Listing provided with the concurrently filed Statement Under 37 C.F.R. §§ 1.821 – 1.825.

Amendments to the claims

1. (Original) A method for treating ischemic heart diseases, which comprises the step of administering angiopoietin-1 or a vector encoding angiopoietin-1.

2. (Original) The method for treating ischemic heart diseases according to claim 1, which comprises the step of administering angiopoietin-1 or a vector encoding angiopoietin-1, and in which a vascular endothelial growth factor is not administered.

3. (Currently Amended) The method according to claim 1 or 2, wherein ~~angiopoietin-1 or~~ the vector encoding angiopoietin-1 is a viral vector ~~encoding angiopoietin-1~~.

4. (Original) The method according to claim 3, wherein the viral vector is an adenoviral vector.

5. (Original) The method according to claim 3, wherein the viral vector is a minus-strand RNA viral vector.

6. (Currently Amended) The method according to claim 1 or 2, wherein ~~angiopoietin-1 or~~ the vector encoding angiopoietin-1 is a naked DNA.

7. (Currently Amended) The method according to any one of claims 1 to 6, wherein ~~angiopoietin-1 or~~ the vector encoding angiopoietin-1 is a vector that drives angiopoietin-1 expression using a CA promoter or a promoter having a transcriptional activity equivalent to or higher than that of said CA promoter.

8. (Original) The method according to any one of claims 1 to 7, wherein the administration of angiopoietin-1 or the vector encoding angiopoietin-1 is an injection into cardiac muscle.

9. (Original) A method for treating ischemic diseases, which comprises the step of administering a viral vector encoding angiopoietin-1.

10. (Original) The method for treating ischemic diseases according to claim 9, which comprises the step of administering a viral vector encoding angiopoietin-1, and wherein a vascular endothelial growth factor is not administered.

11. (Original) The method according to claim 9 or 10, wherein the viral vector is an adenoviral vector.

12. (Original) The method according to claim 9 or 10, wherein the viral vector is a minus-strand RNA viral vector.

13. (Original) The method according to any one of claims 9 to 12, wherein the vector administration is an injection into an ischemic site.

14. (Original) A genetically modified mesenchymal cell comprising a foreign gene encoding angiopoietin-1.

15. (Original) The mesenchymal cell according to claim 14, into which an adenoviral vector encoding angiopoietin-1 has been introduced.

16. (Original) The mesenchymal cell according to claim 14, into which a minus-strand RNA viral vector encoding angiopoietin-1 has been introduced.

17. (Original) A therapeutic composition for ischemia, which comprises the mesenchymal cell according to any one of claims 14 to 16 and a pharmaceutically acceptable carrier.

18. (Original) A method for producing a genetically modified mesenchymal cell, wherein the method comprises the step of contacting the mesenchymal cell with a minus-strand RNA viral vector carrying a gene.

19. (Original) The method according to claim 18, wherein the gene encodes angiopoietin-1.

REMARKS

The title has been amended to describe more accurately the claimed invention. The specification has been amended to cross-reference related applications, to make changes that more accurately reflect the invention, and to correct typographical errors.

Amendment to the specification

The paragraphs starting at page 6, line 17, at page 6, line 22, and at page 6, line 24 have been amended in accordance with changes made to the claims noted below. The paragraph starting at page 17, line 36 has been amended to recite "administration may take place," a change that better describes the present invention and is evident from the context. The paragraph starting at page 24, line 3 has been amended to recite mesodermal "stem" cells. This amendment corrects a typographical error; mesodermal liver cells do not exist. Support for this amendment can be found, for example, at page 23, lines 21-22. The paragraph starting at page 25, line 32, has been amended to recite cord blood "provided by" bone marrow banks, a change that more accurately describes the present invention and better describes the source of cord blood. The paragraph starting at page 28, line 22 has been amended to recite "macrophages" (page 29, line 1) and "dendritic cells" (page 29, line 2). This change corrects a typographical error that is evident from the context; support for these changes can be found, for example, page 28, lines 24-28. The amendment to the paragraph starting at page 30, line 24 alters the text so that it does not state that the amount of plasmid is indicated in Figure 8. The paragraph at page 32,

line 12 has been amended to recite "pAxCAhVEGF" and "pAxCAhAng1," a change that better describes the present invention and is evident from the context. The paragraphs starting at page 35, line 20, at page 35, line 33, and at page 36, line 4 have been amended to recite "rat" GAPDH primer. This change evident from the context; support for this change is found, for example, at page 35, lines 30-31. The paragraph at page 37, line 20 has been amended to recite "diameters." This change better describes the invention; applicants note that it is convention in the art to measure a blood vessel by its diameter. The amendment to the paragraphs starting at page 38, line 23, at page 38, line 26, and at page 38, line 33 corrects English language usage and grammar. The paragraph starting at page 43, line 10 has been amended to recite "1x 10^{9}" and hind leg "skin." The change at page 43, line 13 (1x 10^{9}) generates consistent formatting. The change at page 43, line 14 (hind leg skin) better describes the experimental procedure, and is evident from the context. The paragraph starting at page 44, line 21 has been amended to recite "1x 10^{9}," a change that generates consistent formatting. The paragraph starting at page 44, line 28 has been amended to recite "into the cardiac muscle." This change better describes the experiment performed; support for this amendment can be found, for example, in the description of Figure 9 at page 30, lines 29-32. The paragraph starting at page 47, line 33 has been amended to recite the "SeV" vector overflow. This change is evident from the context; support for this change can be found, for example, at page 47, line 9 and page 47, lines 34-36. The amendment to the paragraphs starting at page 49, line 11 and page 49, line 14 corrects English language usage. The paragraph starting at page 50, line 3 has

been amended to recite the left iliac artery and the left femoral artery. Support for this change can be found, for example, at page 50, lines 5-6, which recites the left inguinal region, and at page 50, lines 9-10. The paragraph starting at page 50, line 15 has been amended to recite day "14" after ischemia. This correction is evident from the context, and support for this change can be found, for example, at page 50, line 16-17, which recites two weeks after ischemia. The paragraph starting at page 51, line 8 has been amended to recite rat mesenchymal stem cells. This change corrects a typographic error and is evident from the context. Support for this change can be found, for example, at page 51, line 17 and page 51, line 19. Finally, the paragraph starting at page 53, line 1 has been amended to recite "human cervical cancer cell." This change is evident from the context; applicants note that HeLa cells, which are described at page 53, line 3, are human cervical cancer cells, as is known in the art. These changes add no new matter.

A replacement Sequence Listing updating fields 110-170 has been submitted. No new matter has been added by the present amendment.

Amendment to the claims

Claim 3 has been amended to recite a viral vector encoding angiopoietin-1. In view of the amendment to claim 3, claims 6 and 7 have also been amended. No new matter is added by this amendment.

COPY

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 18 AUGUST 2005

James D. DeCamp, Ph.D.
Reg. No. 43,580

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045